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# STRUCTURE OF WATER IN *ESCHERICHIA COLI* B

Christopher S. Cox
Harold Klapper

JUNE 1970



DEPARTMENT OF THE ARMY
Fort Detrick
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# STRUCTURE OF WATER IN ESCHERICHIA COLI B

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BIOLOGICAL SCIENCES LABORATORIES

Project 1T061101A91A June 1970

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ABSTRACT

Nuclear magnetic resonance (NMR) was used to measure the degree of order of water in Escherichia coli B. It was necessary to grow the E. coli B in a special chemically defined medium, which contained very low concentrations of paramagnetic ions (about $10^{-6}$ moles per liter). Complex media or general chemically defined media gave bacteria with such high paramagnetic ion concentrations that it was difficult to differentiate between paramagnetic ion broadening and water structure broadening of the NMR water spectrum. Using bacteria grown in the special chemically defined medium, we showed that the water in E. coli B was highly ordered and was very different from "free" water and from polywater.

STRUCTURE OF WATER IN ESCHERICHIA COLI B*

Cox[1,2] has shown that aerosol survival of Escherichia coli B is only approximately related to water content of the organism. Nuclear magnetic resonance (NMR) was used to determine the structure of water in the organism at different water contents. This technique has been employed to determine water structure with Nocardia asteroides by Cerbón,[3] skeletal muscle by Hazlewood, Nichols and Chamberlain,[4] and muscle and brain by Cope.[5] NMR is suited to the task because the width of the signal produced by water hydrogens is dependent upon the motional freedom of the water molecules.[6,7] As mobility of the water molecules increases, line width decreases. In the present work the NMR spectra were obtained with a Varian A60 spectrometer.

In initial experiments, Escherichia coli B were grown in a complex tryptone medium, using frozen inocula.[8] The bacteria were harvested by centrifugation after 16 hours' growth at 37 C and were packed into the bottom of an NMR tube. The NMR spectra of water in the bacteria showed considerable line broadening compared with a control of "free" distilled water. The position of the peak was shifted to a higher magnetic field strength. It was thought possible that the broadening could have been caused by paramagnetic ions in the bacteria. Therefore, using atomic absorption spectra, analyses were made for Fe, Mn, and Cu in the bacteria. The following values were obtained: $2.5 \times 10^{-2}$ moles per liter of Fe, $7.5 \times 10^{-5}$ moles per liter of Mn and $1 \times 10^{-5}$ moles per liter of Cu. NMR spectra were then run for solutions of these ions at the above concentrations. $Mn^{++}$ and $Cu^{++}$ caused negligible effects, but $Fe^{+++}$ caused appreciable line broadening. However, the broadening was less than that for the bacteria and no shift of the peak to higher magnetic field strengths occurred. Hence, E. coli B grown in a complex tryptone medium were not really suitable for the present work. We therefore decided to use the chemically defined medium previously described,[9] but to omit the ferric ammonium citrate and treat the medium in the manner described below.

It was known that hard centrifugation of bacteria grown in 100 ml of the chemically defined medium produced a pellet of approximately 1 ml. Assuming that the bacteria had accumulated all of the ion under consideration, then there was an effective concentration of 100-fold. It follows that, to have bacteria with an ion content of $1 \times 10^{-4}$ moles per liter, the medium (before inoculation and growth) must contain no more than $1 \times 10^{-6}$ moles per liter of that ion. Analysis and calculation showed that the concentration of Fe in the chemically defined medium[9] caused by impurities in the components would be greater than $1 \times 10^{-6}$ moles per liter. Therefore, the following procedure was adopted. To each 100-ml sample of chemically defined media was added 20 ml of 1% 8-hydroxyquinoline in chloroform; the mixture was shaken for 1 min in a separation funnel and

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the chloroform phase was removed. The process was repeated four more times, then the aqueous phase was shaken for 1 min with 20 ml of chloroform to extract the 8-hydroxyquinoline dissolved in the aqueous phase. After this treatment the following were added to give final concentrations of $1 \times 10^{-7}$ moles per liter: Cd, Co, Cu, Mo, Mn, Ni, and Zn. $Fe^{+++}$ was also added at a final concentration of $2 \times 10^{-6}$ moles per liter. The medium was then autoclaved to sterilize it and to remove the chloroform. After the addition of a very small inoculum and growth for 24 hours at 37 C, the bacteria were harvested by centrifugation. Analysis by atomic absorption spectroscopy showed the following ion contents: Fe = $3.4 \times 10^{-4}$ moles per liter, Mn = $9.8 \times 10^{-6}$ moles per liter, Cu = $3.6 \times 10^{-5}$ moles per liter. These agree quite well with the theoretical values, assuming that the bacteria accumulated all of the respective ions. It was established that such concentrations of paramagnetic ions had little influence on the line width of the NMR spectra of water.

The NMR spectrum of water in E. coli B grown in the above manner is shown in Figure 1, which includes the NMR spectra of "free" water and tetramethylsilane (TMS). It is evident that the NMR spectra of water in E. coli B is much broader than "free" water and that it is shifted upfield. These results indicate that the structure of water in E. coli B is more ordered than that in "free" distilled water. It is possible that if E. coli B contained appreciable amounts of free radicals, then these might have caused line broadening. However, it has been shown that Serratia marcescens[10,11] and E. coli B[12,13] do not contain detectable free radicals in the dry state in the absence of oxygen, or when wet even in the presence of oxygen.[10,11]

It was of interest to determine the NMR spectrum of the water contained in the bacteria when the water content of the bacteria was reduced. This was achieved by freeze-drying the bacteria and equilibrating over sulfuric acid solutions under nitrogen to prevent possible free radical formation caused by the presence of oxygen. When equilibrated with water vapor of activity 0.962, the water in the bacteria gave an NMR spectrum very like that in Figure 1, although broader. Reducing the water activity to 0.852 (which corresponds to a region where aerosol survival is particularly poor[1,2,14]) caused the NMR signal of the water to become so broad that it could not be differentiated from the background, even by using a computer of average transients. It was established that if the amount of water in the sample had been "free," then it would have very readily been detected by NMR. One of the problems of these types of experiments is that when the water activity of the bacteria is reduced, the bacteria shrink. It is extremely difficult to allow for this effect and for the reduction of water activity in terms of the activity of the paramagnetic ions, and hence their contribution to line broadening. It is suggested, however, that this contribution would not be sufficient to cause the observed results, because the NMR spectrum of $2.5 \times 10^{-2}$ moles per liter of Fe is not as broad as that for the bacteria at a water activity of 1.0.

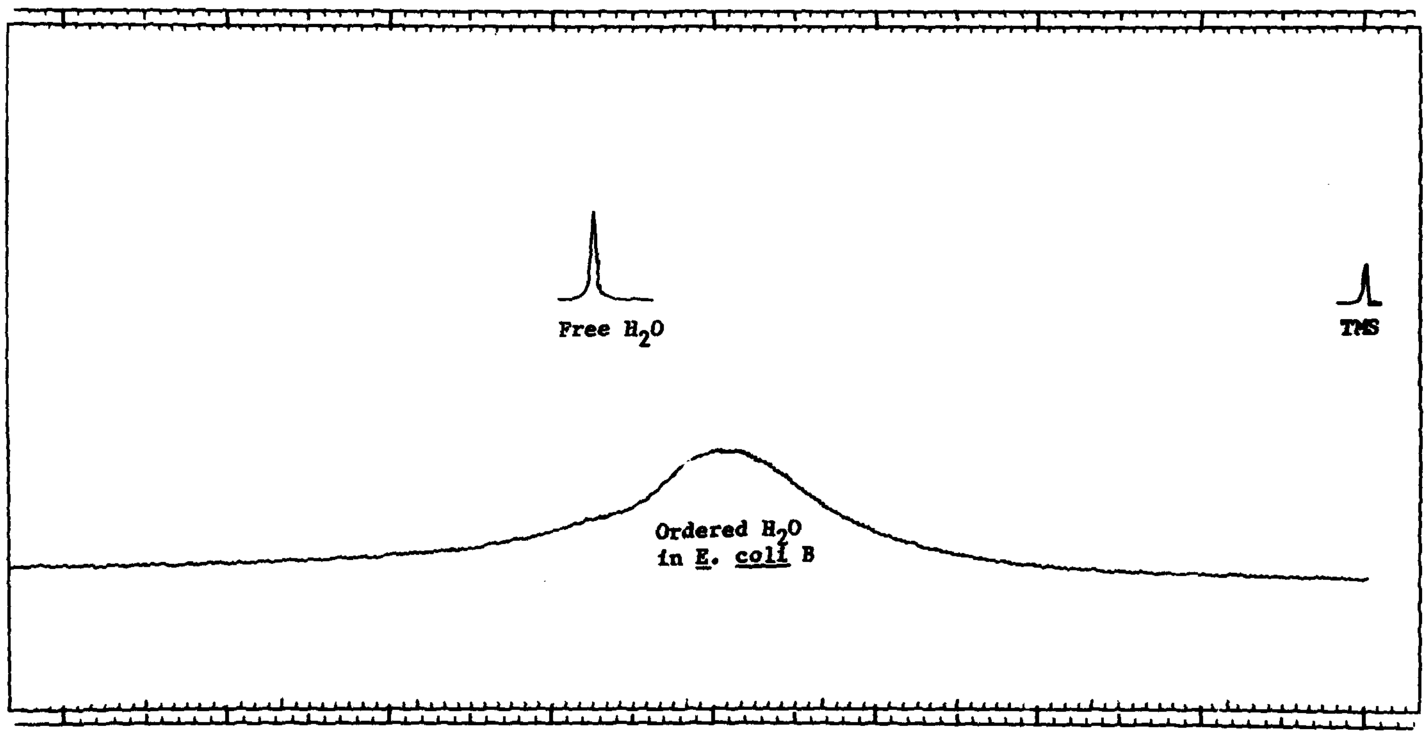


FIGURE 1. The NMR Spectrum of Water in _Escherichia coli_ B; Water and Tetramethylsilane Standards are Included.

Pertinent to this study was an examination for polywater[15] in E. coli B, since the NMR spectrum of polywater was known. Polywater produces a broad NMR signal about 300 Hz downfield from normal "free" water.[16]* The NMR signal of the water in E. coli B was examined in this region, but no signal due to polywater could be detected even by using a computer of average transients. Hence, either E. coli B does not contain polywater in any appreciable quantity or, if it does, it is even more ordered than "free" polywater, so that the NMR signal cannot be differentiated from background, even with a computer of average transients. In any case, the data do show that E. coli B contains ordered water that is different from polywater and from "free" water. The data also suggest that the water in E. coli B becomes more ordered as its activity decreases. Such a result agrees with sorption isotherm data.

* Page, T.; Jacobsen, R.; Lippincott, E.R. 1969. Personal communication.

## LITERATURE CITED


1. Cox, C.S. 1968. The aerosol survival of *Escherichia coli* B in nitrogen, argon and helium atmospheres and the influence of relative humidity. J. Gen. Microbiol. 50:139-147.

2. Cox, C.S. 1970. Aerosol survival of *Escherichia coli* B disseminated from the dry state. Appl. Microbiol. 19:604-607.

3. Cerbón, J. 1964. Nuclear magnetic resonance of water in microorganisms. Biochim. Biophys. Acta 88:444-447.

4. Hazlewood, C.F.; Nichols, B.L.; Chamberlain, N.F. 1969. Evidence for the existence of a minimum of two phases of ordered water in skeletal muscle. Nature 222:747-750.

5. Cope, Freeman W. 1969. Nuclear magnetic resonance evidence using $D_2O$ for structured water in muscle and brain. Biophys.J. 9:303-319.

6. Kavanau, J.L. 1964. Water and solute-water interactions. Holden-Day, Inc., San Francisco, California.

7. Emsley, J.W.; Feeney, J.; Sutcliffe, L.H. 1965. High resolution nuclear magnetic resonance spectroscopy. Pergamon Press, New York, N.Y.

8. Cox, C.S. 1968. Method for the routine preservation of micro-organisms. Nature 220:1139.

9. Cox, C.S. 1969. The cause of loss of viability of airborne *Escherichia coli* K12. J. Gen. Microbiol. 57:77-80.

10. Heckly, R.J.; Dimmick, R.L.; Windle, J.J. 1963. Free radical formation and survival of lyophilized microorganisms. J. Bacteriol. 85:961-966.

11. Heckly, R.J.; Dimmick, R.L. 1968. Correlations between free radical production and viability of lyophilized bacteria. Appl. Microbiol. 16:1081-1085.

12. Lion, M.B.; Kirby-Smith, J.S.; Randolph, M.L. 1961. Electron spin resonance signals from lyophilized bacterial cells exposed to oxygen. Nature 192:34-38.

13. Cox, C.S.; Baldwin, F. 1967. The toxic effect of oxygen upon the aerosol survival of *Escherichia coli* B. J. Gen. Microbiol. 49:115-117.

14. Cox, C.S. 1966. The survival of Escherichia coli atomized into air and into nitrogen from distilled water and from solutions of protecting agents, as a function of relative humidity. J. Gen. Microbiol. 43: 383-399.

15. Lippincott, E.R.; Stromberg, R.R.; Grant, W.H.; Cessac, G.L. 1969. Polywater. Science 164:1482-1487.

16. Petsko, G.A. 1970. Proton magnetic resonance spectrum of polywater. Science 167:171.

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13. ABSTRACT

Nuclear magnetic resonance (NMR) was used to measure the degree of order of water in *Escherichia coli* B. It was necessary to grow the *E. coli* B in a special chemically defined medium, which contained very low concentrations of paramagnetic ions (about $10^{-6}$ moles per liter). Complex media or general chemically defined media gave bacteria with such high paramagnetic ion concentrations that it was difficult to differentiate between paramagnetic ion broadening and water structure broadening of the NMR water spectrum. Using bacteria grown in the special chemically defined medium, we showed that the water in *E. coli* B was highly ordered and was very different from "free" water and from polywater.

14. Key Words

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Polywater

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